AbdelHamid et al. *SECI Oncology* 2015
DOI: 10.18056/seci2015.6

Impact of the Clinicolaboratory Characteristics on the Treatment Outcome of the Pediatric Patients with Acute Lymphoblastic Leukemia at South Egypt Cancer Institute

Omnia N. AbdelHamid[1], Amany M. Ali[1], Douaa M. Sayed[2], Mohammed M. Ghazaly[3]

[1] Pediatric Oncology Department, South Egypt Cancer Institute, Assiut University.
[2] Clinical Pathology Department, South Egypt Cancer Institute, Assiut University.
[3] Department of Pediatrics, Faculty of Medicine, Assiut University

Correspondence should be addressed to Omnia N. AbdelHamid at Pediatric Oncology Department, South Egypt Cancer Institute, El-Methak St., Asyut, Egypt, o.nagy_hamid@yahoo.com

Published 11 June 2015

Abstract

BACKGROUND: In pediatric acute lymphoblastic leukemia (ALL), patients are treated according to risk groups defined by both clinical and laboratory features. We aimed at the identification of the nature of the clinical and laboratory features that our pediatric ALL patients possess at presentation and the determination of the impact of these features on the survival rates of them.

METHODS: from January 2008 till January 2014, 172 pediatric patients (102 boys and 70 girls, age 2-15 years old) with newly diagnosed ALL who presented at the Pediatric Oncology Department, South Egypt Cancer Institute were analyzed for treatment outcome, disease free- and event free survival rates according to the presenting clinical and laboratory features. After being submitted for the standard diagnostic workup, the patients were assigned into standard and high risk groups. The response to treatment was estimated at the specified time points of the treatment protocol. The treatment outcome, overall - and event free survival rates were statistically calculated at the end of the study.

RESULTS: favorable age group (2- < 10 years old) represented 76.2%. Mediastinal adenopathy and bulky extramedullary disease represented 9.9% and 14% respectively. Central nervous system (CNS) involvement with leukemia was encountered in 3.5% of the patients. Favorable initial WBC count (< 50.000 x109/μL) was found in 70.9%, and B-cell phenotype in 80.8% of patients. We found that favorable age, absence of certain clinical features as bulky disease; mediastinal adenopathy and CNS leukemia and presentation with favorable WBC count were all associated with remarkably higher remission rate and significantly lower relapse rate. DFS rate was significantly higher for patients presented at favorable age and for those who had no bulky disease or mediastinal adenopathy at presentation.

CONCLUSION: this study showed that presentation with bulky extramedullary disease and mediastinal adenopathy can confer a negative impact on the remission rate and survival rates of the pediatric ALL patients treated at Pediatric Oncology Department, South Egypt Cancer Institute. It also showed that gender of the patients has no remarkable impact on the outcome of these patients.

Keywords: acute lymphoblastic leukemia, bulky extramedullary disease, mediastinal adenopathy.

Background:

Acute lymphoblastic leukemia (ALL) is the most common malignancy in the pediatric age group with incidence of 29.2/1000000 children in the United States (1). In Egypt, ALL represented 50.9% of total pediatric leukemia during 2009 as reported by El-Minia Cancer Center (2). In our department, ALL accounted for 29.3% of total malignancies and 87.8 % of total Leukemia cases (3). With current treatment strategies, around 80% of children with ALL achieve long-term remission (4-9). Many pediatric ALL groups adopted the principle of risk based treatment to minimize treatment intensity in patients with low risk criteria and preserve the intensive treatment options for patients with high risk criteria (4,10). Presenting age and WBC count are the simplest features that determine the risk assignment of the pediatric patients with ALL (11). Other features as CNS status, bulky disease, mediastinal

adenopathy and immunophenotype are also prognostic (6,8,10,12,13). Periodic assessment of treatment response provides an indication of the sensitivity of leukemic cells to chemotherapy (14), so accurate and sensitive determination of treatment response i.e. minimal residual disease (MRD) is a powerful predictor of clinical outcome in childhood ALL (13,15). In the current study, we aimed at evaluating the impact of the presenting features on the treatment outcome, overall and disease free survival rates of children with de novo ALL who presented at the Pediatric Oncology Department, South Egypt Cancer Institute, from January 2008 to January 2014. .

Methods:

From January 2008 to January 2014, 172 pediatric patients with de novo ALL, 2-15 years old were prospectively analyzed for treatment outcome and survival rates according to the presenting clinical and laboratory features. The study was approved by the local ethical committee and informed consent was taken from the patients' guardians. All patients underwent the standard diagnostic workup of ALL. The diagnosis of ALL was based on both the morphologic and cytochemical examination of bone marrow (BM) smears as well as on the immunophenotyping (IPT) of the leukemic blast cells using multicolor flow cytometry. Lumbar puncture for cerebrospinal fluid (CSF) examination to determine the CNS status was also done for all patients. The CNS status of each patient was defined as follows: (i) CNS1, no identifiable blast cells in a non-traumatic CSF; (ii) CNS2, < 5 WBC/μL with identifiable blast cells in a non-traumatic CSF; (iii) CNS3, ≥ 5 WBC/μL with identifiable blast cells in a non-traumatic CSF or the presence of cranial nerve palsy (3,16). A traumatic lumbar puncture (≥10 erythrocytes/μL) that includes blasts at diagnosis has also been associated with increased risk of CNS relapse (17), Some clinical trial groups have approached CNS2 and traumatic lumbar puncture by utilizing more intensive therapy, primarily additional doses of intrathecal therapy during induction (18). Other groups have not altered therapy based on CNS2 status (19). Bulky extramedullary disease was defined by either: a mediastinal mass > 33% of the intrathoracic dimension at the level of the fifth thoracic vertebra, lymphadenopathy > 3 cm in dimension for a single lymph node or > 5 cm in dimension for a group of contiguous lymph nodes, or splenomegaly in the supine position with the spleen tip palpated below the umbilicus (20).

Risk Classification: Patients were stratified as standard risk (SR) or high risk (HR) based on the COG ALL Risk Classification System (7,9,13). SR criteria were B-cell precursor with age 2- < 10 years at presentation, WBC count < 50x109/μL, and CNS1 status. All other patients were classified as HR.

Treatment Protocol: The patients were treated on the modified TXIIIB protocol adopted from St. Jude Children Research Hospital (SJCRH) (Table 1) (14). We extended the continuation treatment for all the males to be 146 weeks (total 3 years of chemotherapy) as recommended by many pediatric ALL groups (1,11,15). The extended weeks constituted of monthly vincristine (1.5 mg/m2) intervened by weekly intramuscular methotrexate (40 mg/m2) and daily mercaptopurine (60 mg/m2).

Response and Relapse Criteria: At the end of induction phase, full clinical assessment and BM aspiration (BMA) for cytomorphologic assessment of remission were done for all patients. Complete remission was defined as the absence of leukemic blasts in peripheral blood and CSF and less than 5% blasts on BMA smear and no evidence of extramedullary disease (1,11). Relapse was defined as recurrence of ≥ 25% lymphoblasts in BM or localized leukemic infiltrates at any site (1,11).

Statistical Analysis: Overall Survival (OS) was defined as the length of time from the date of diagnosis that patients are still alive. Disease Free survival (DFS) was defined as the length of time that the patient survives in clinical and laboratory remission from the date of diagnosis. Cox regression analysis was done for multivariate estimation of the impact of the different clinical features on the overall survival and DFS.

Results:

The mean duration of follow up of the patients accrued to our study was 19.1±13.8 months (range 0.5-56.7 months). The patients' characteristics and the treatment outcome were as follows:

Patients' Characteristics: The presenting clinical and laboratory characteristics of the 172 patients are reported in Table 2. The median age at diagnosis was 5 years (range 2 to 15 years) and male to female ratio was 1.45:1.

Treatment Outcome: Of the total 172 patients; 113 (65.7%) continued in remission, 26 relapsed (18 isolated medullary, 6 isolated CNS and 2 combined medullary and CNS relapses), 19 died in remission (18 from sepsis and one from methotrexate toxicity) and 14 abandoned the treatment. The 4-year overall (OS), disease free- (DFS) and event-free (EFS) survival rates (±SE) were 89% (±2), 83% (±2.5) and 73.8% (±2.7) respectively (Figure 1).

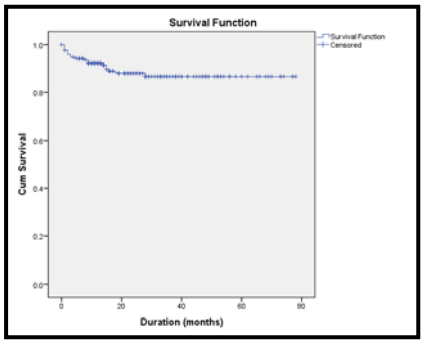
Prognostic Clinical Characteristics: On analysis of the impact of different clinical and laboratory characteristics on the treatment outcome, OS and DFS rates, we found that age, bulky disease, mediastinal adenopathy and CNS status all had significant effect on the treatment outcome and survival rates (Table 2, Figure 2).

Table 1. Total Therapy XIIIB Protocol Used For The Treatment Of The Newly Diagnosed Pediatric ALL Patients At POD, SECI (Modified From TXIIIB Protocol Adopted From SJCRH).

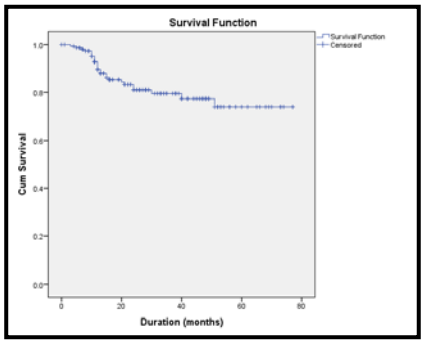
Drug (s)	Dose	Schedule
1) Up-Front Therapy:		
Prednisone	40 mg/m^2 /day PO	Days 1-4
2) Remission Induction:		
• **Prednisone**	40 mg/m^2/day PO	Days 1-28
• **Vincristine**	1.5 mg/m^2/day IV	Days 1, 8, 15, 22
• **Daunorubicin**	25 mg/m^2/day IV infusion	Days 1 and 8
• **L-asparaginase**[$]	10.000 U/m^2/day IM	Days 2, 4, 6, 8, 10, 12
• **Etoposide**	300 mg/m^2/day IV infusion	Days 22, 25, 29
• **Cytarabine**	300 mg/m^2/day IV	Days 22, 25, 29
• **Intrathecal (TIT)***	age-appropriate	Days 5 and 26
3) Consolidation Therapy:		
• **High-dose methotrexate**	2 g/m^2 over 2 hours IV infusion	2 weekly doses
• **Leucovorin Rescue**	10 mg/m^2/6 hours IV	At hours 44, 50, 56, 62, 68 from the start of HDMTX infusion.
• **Mercaptopurine**	75 mg/m^2/night PO	For 2 weeks
4) Post-remission Therapy for Lower-Risk ALL (120 weeks):		
• **Weekly methotrexate 40 mg/m^2 IV + mercaptopurine 75 mg/m^2/night PO.**		
• **The weekly therapy is reinforced during the first year by:**		
o **Dexamethasone (8 mg/m^2/day for 7 days) + vincristine (1.5 mg/m^2) every 4 weeks.**		
o **High-dose methotrexate and mercaptopurine (as consolidation therapy) every 8 weeks.**		
• **Re-induction (weeks 16 to 21): similar to the initial 4-week induction, one dose of etoposide + cytarabine on day 22, followed by 2 weekly doses of consolidation therapy.**		
5) Post-remission Therapy for Higher-Risk ALL (120 weeks in weekly rotation):		
<u>Week 1.</u> Etoposide 300 mg/m^2 IV + cyclophosphamide 300 mg/m^2 IV		
<u>Week 2.</u> Methotrexate 40 mg/m^2 IV + mercaptopurine 75 mg/m^2/night PO.		
<u>Week 3.</u> Methotrexate 40 mg/m^2 IV + cytarabine 300 mg/m^2 IV		
<u>Week 4.</u> Vincristine 1.5 mg/m^2 IV + dexamethasone 8 mg/m^2/day PO in 3 divided doses		
<u>Week 5.</u> Etoposide 300 mg/m^2 IV + cyclophosphamide 300 mg/m^2 IV		
<u>Week 6.</u> Methotrexate 2 g/m^2 IV over 24 hours + mercaptopurine 75 mg/m^2/night PO		
<u>Week 7.</u> Etoposide 300 mg/m^2 IV + cytarabine 300 mg/m^2 IV		
<u>Week 8.</u> Vincristine 1.5 mg/m^2 IV + dexamethasone 8 mg/m^2/day PO in 3 divided doses.		
• **Re-induction (weeks 16 to 21): similar to lower-risk ALL**		
6) CNS Directed Therapy		

[$]added doses on days 15, 17, 19 in patients with 5% or more blasts in day-15 marrow.
[*] 2 additional TIT for patients with CNS2 and CNS3 on days 12 and 19.
PO: per os (oral), IV: intravenous, IM: intramuscular, TIT: triple intrathecal, HDMTX: high dose methotrexate

(A)



(B)



(C)

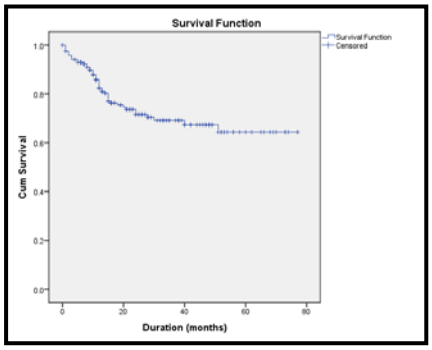
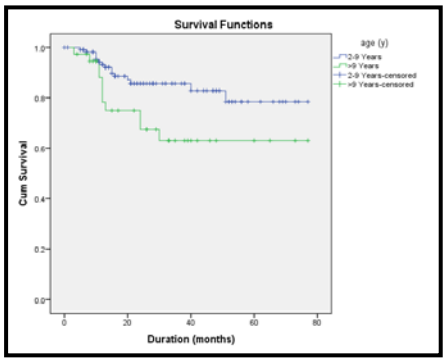
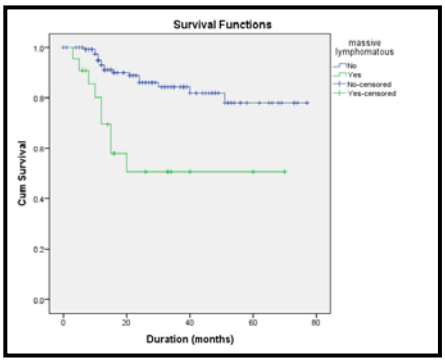


Figure 1: Kaplan Meier Plot for (A) overall, (B) disease free and (C) event free survival rates for all the 172 pediatric ALL patients

(A)



(B)



(C)

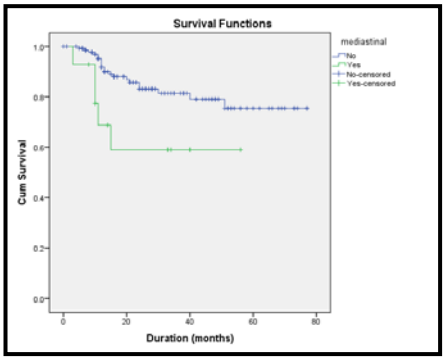


Figure 2: Kaplan Meier Plot for disease free survival rate of (A) age (favorable vs. unfavorable) (P 0.03), (B) bulky disease (absent vs. present) (P 0.001) and (C) mediastinal adenopathy (absent vs. present) (P 0.03) for all the 172 pediatric ALL patients.

Table 2. Treatment Outcome, OS and DFS Rates Of The 172 Pediatric ALL Patients Treated According to the Modified TXIIIB Protocol at the Pediatric Oncology Department, South Egypt Cancer Institute in Relation to the Selected Clinical and Laboratory Features

Clinical Characteristic	Patients (%) N= 172	CCR No. (%) N= 113	P value	Relapse No. (%) N= 26	P value	DFS % (±SE)	P value
Age (years):							
• 1 - <10	131 (76.2)	89 (67.9)	0.3	15 (11.5)	**0.02**	87 (±2.7)	**0.03**
• ≥ 10	41 (23.8)	24 (58.5)		11 (26.8)		71.1 (±5.5)	
Gender:							
• Boys	102 (59.3)	66 (64.7)	0.7	16 (15.7)	0.8	82.2 (±3.4)	0.6
• Girls	70 (40.7)	47 (67.1)		10 (14.3)		84.1 (±4)	
Bulky Disease:							
• Present	25 (14.5)	13 (52)	0.1	9 (36)	**0.001**	59.1 (±7)	**0.001**
• Absent	147 (85.5)	100 (68)		17 (11.6)		87 (±2.6)	
Mediastinal Adenopathy:							
• Present	17 (9.9)	8 (47.1)	0.08	5 (29.4)	0.08	64.3 (±2.6)	**0.03**
• Absent	155 (90.1)	105 (67.7)		21 (13.5)		84.9 (±6.6)	
CNS Status:							
• CNS-3	6 (3.5)	2 (33.3)	0.09	3 (50)	**0.02**	75 (±12.2)	0.8
• CNS-1	166 (96.5)	111 (66.9)		23 (13.9)		83.2 (±2.6)	
WBC ($x10^9/mm^3$):							
• <50	122 (70.9)	84 (68.9)	0.17	16 (13.1)	0.2	85.2 (±2.9)	0.2
• ≥50	50 (29.1)	29 (58)		10 (20)		77.8 (±4.5)	
Immunophenotyping:							
• B-cell	139 (80.8)	93 (66.9)	0.5	17 (12.2)	0.15	87.5 (±4)	0.09
• T-cell	33 (19.2)	20 (60.6)		9 (27.3)		70 (±5.6)	

CCR: continuous complete remission, DFS: disease free survival, EFS: event free survival, SE: standard error, CNS: central nervous system, WBC: white blood cell count, mm3: cubic milliliter, N: number.
NB. The data of the patients who died in remission and those who abandoned treatment is not shown.
P value was calculated using the Chi square test.

Discussion:

Certain clinical and laboratory characteristics at presentation in pediatric ALL were significantly predictive of treatment outcome and survival rate. In the current study, we tried to determine the features that predict the outcome of the pediatric patients with de novo ALL who presented to our department at South Egypt Cancer Institute from January 2008 to January 2014. The age at presentation was the simplest clinical criterion that can be used to judge the prognosis of the patients (13). Patients with favorable age group (2 - < 10 years) showed significantly higher DFS (87% vs. 71.1% P 0.03) and lower relapse rates (11% vs. 26.8% P 0.02) than patients with unfavorable age group (≥ 10 years). Favorable age at presentation is usually associated with better outcome and survival rates than the unfavorable age owing to its association with favorable biologic features (22,23) while unfavorable age is known to be associated with higher WBC count, T-cell phenotype and unfavorable biologic features (24). However, the treatment outcome of the patients who present at age ≥ 10 years old had improved dramatically over years owing to the exploitation of the

more intensive pediatric chemotherapy regimens rather than the adult ones (25-28).

Female gender was associated with slightly higher but insignificant continuous complete remission (67.1% vs. 64.7% in males P 0.7), DFS and EFS rates (84% vs. 82.2% and 75.7% vs. 72.5% in males P 0.6 and 0.5 respectively than males). Male gender was also associated with higher relapse rates (15.7% vs. 14.3% in girls P 0.8). Some studies still show that the prognosis of ALL in girls is slightly better than for boys owing to the higher testicular relapse rate in boys (29-31) while other studies showed that the prognosis of boys was approaching that of girls (19). Differences in drug metabolism secondary to polymorphisms in enzyme involved in xenobiotic metabolism in boys contribute to their sensitivity to anti-leukemic drugs (32).

Our patients who presented with bulky disease showed a significantly higher relapse rate (36% vs. 11.6% P 0.001) and lower DFS rate than those who had no bulky features (59% versus 87% P 0.001) (table 2) owing to its association with other aggressive risk factors as unfavorable age, T-cell phenotype, mediastinal adenopathy and slower response to chemotherapy as we found in our study.

For patients who presented mediastinal adenopathy, we encountered a relatively higher percentage of mediastinal adenopathy at presentation (9.9%) than other studies (33,34) and those patients showed significantly lower DFS rate (64.3% vs. 84.9% P 0.03) than those who had no mediastinal adenopathy at presentation. They also had remarkably lower but insignificant continuous remission rate (47.1% vs. 67.7% P 0.08) and higher relapse rate (29.4% vs. 13.5% P 0.08) than those who had no mediastinal adenopathy at presentation. The less favorable outcome can be partly attributed to the association of mediastinal adenopathy with other aggressive prognostic features including older age, high initial WBC count, T-cell phenotype and bulky disease. Möricke et al., 2010 showed that, in BFM-86 trial, mediastinal adenopathy at presentation was a criterion for high risk assignment but not in the subsequent trials while Attarbaschi et al., 2002 reported that mediastinal adenopathy as well as its rate of regression lack prognostic significance.

The presence or absence of CNS leukemia (CNS3) at diagnosis has prognostic significance, as these patients are at a higher risk of treatment failure (both within the CNS and systemically) owing to its association with other aggressive risk factors and bad biologic features (37-39). CNS is considered as a sanctuary site and both high dose systemic chemotherapy as well as CNS directed therapy are necessary in order to control the CNS disease (40,41). The presence of CNS leukemia in the ALL patients who accrued to our study was associated with a significantly higher relapse rate (50% vs. 13.9% P 0.02) than patients with no CNS leukemia.

WBC count at diagnosis was incorporated in the first risk stratification of pediatric patients with ALL at diagnosis and a count of 50,000/μL is generally used as an operational cutoff point between better and poorer prognosis (12). Patients with unfavorable WBC count (≥ 50.000/μL) at diagnosis showed a remarkable but insignificant higher relapse rate (20% vs. 13.1% P 0.2), lower remission rate (58% vs. 68.9% P 0.17) and lower DFS (77.8% vs. 85.2% P 0.2) rate than those with favorable presenting WBC count (<50.000/μL). The insignificant results are related to the short duration of follow up of the patients in this study (median duration 19 months). Another contributing factor to the inferior outcome of these patients was the association of unfavorable WBC count at diagnosis with other aggressive risk factors as T-cell phenotype, bulky extramedullary disease, mediastinal adenopathy and CNS leukemia. Vaitkevičienė et al., 2011 showed that high WBC at diagnosis was a modifying factor for the treatment outcome of the patients with B-cell phenotype but not for those with T-cell phenotype and is associated with a slower end induction remission rate.

The pediatric ALL patients B-cell phenotype included in this study had slightly and insignificant higher continuous complete remission rate than those with T- cell phenotype (66.9% vs. 60.6% P 0.5). We encountered higher percentage of pre-B cell (58.7%) subtype which -in some reports- is associated with t(1;19) in 25% of the occasions which requires more intensive treatment and confers inferior outcome (43). Also, the outcome of the patients with B-cell ALL can be modified by age and WBC count at diagnosis, presence of CNS leukemia (18,43,37-39) and presence of other unfavorable biologic features (35,42,44). By analysis, around 67.6% of our patients with B-cell phenotype had one or more unfavorable clinical or laboratory features that contributed to the poor outcome. However, patients with T-cell phenotype showed markedly but insignificant higher relapse rate (27.3% vs. 12.2% P 0.15) and lower DFS rate (70% vs. 87.5% P 0.09) than those with B-cell phenotype. Gaynon et al., 2010 and Möricke et al., 2010 reported higher relapse rate and lower both DFS and EFS rates for patients with T-cell phenotype and attributed this inferior outcome to the its association with other aggressive prognostic factors as older age, higher initial WBC count, bulky extramedullary disease and mediastinal adenopathy. They also reported that with appropriately intensive therapy, T-cell ALL have an outcome approaching that of B-cell ALL.

Conclusion:

Certain clinical and laboratory criteria at diagnosis of pediatric patients with ALL can have a significant impact on the treatment outcome and survival rates of these children. Of these, are the presentations with bulky extramedullary disease and/or mediastinal adenopathy and presence of CNS leukemia necessitating aggressive treatment. WBC count at diagnosis is independent risk factor that can modify the outcome of other factors. Gender of the patient has no longer a significant impact on the treatment outcome and survival of the pediatric patients with ALL.

List of Abbreviations:

BMA: Bone marrow aspirate.
CNS: Central Nervous System.
CSF: Cerebrospinal Fluid.
DFS: Disease Free Survival Rate.
HLA: Human Leucocytic Antigen.
HR: High risk.
OR: Overall Survival Rate.
SJCRH: St. Jude Children Research Hospital.
SR: Standard risk.
TXIIIB Total Therapy XIIIB.
WBC: White blood cell.
µL: Microliter.

Competing Interests:

Authors declare that they have no significant competing financial, professional or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

Acknowledgement:

The authors thank Dr. Nabiel NH Mikhail for his contribution in the statistics of this study.

References:

1) Margolin F. Judith, Rabin Karen R, Steuper C. Philip, Poplack David G. "Acute Lymphoblastic Leukemia". **Principles and Practice of Pediatric Oncology.** Ed. Philip A. Pizzo, Ed. David G. Poplack. Philadelphia: Lippincott Williams and Wilkins, 2011. 518-565. Print.
2) Ibrahim AS, Mikhail NNH, Saber R, Afifi H, Baraka H, Khaled H, Abdel Wahed S, Abdel Latef AS: **Egypt National Cancer Registry: El-Minia Profile 2009**. Egypt National Cancer Registry, 2011 (In Press).
3) Ibrahim AS, Mikhail NN, Saber R, Afifi H (Eds.), **Egypt National Cancer Registry, El-Minia Profile; 2009.**
4) IT Center SECI: incidence of pediatric ALL.
5) Smith MA, Altekruse SF, Adamson PC, Reaman GH, Seibel NL. **Declining childhood and adolescent cancer mortality. Cancer** 2014;**120**:2497-506. DOI: 10.1002/cncr.28748.
6) Aricò M, Valsecchi MG, Rizzari C, Barisone E, Biondi A, Casale F, Locatelli F, Nigro LL, Luciani M, Messina C, Micalizzi C, Parasole R, Pession A, Santoro N, Testi AM, Silvestri D, Basso G, Masera G, Conter V. Long term results of the AIEOP-ALL-95 **Trial for Childhood Acute Lymphoblastic Leukemia: insight on the prognostic value of DNA index in the framework of Berlin-Frankfurt-Muenster based chemotherapy**. J Clin Oncol. 2008;**26**(2):283-289. DOI: 10.1200/JCO.2007.12.3927
7) Möricke A, Reiter A, Zimmermann M, Gadner H, Stanulla M, Dördelmann M, Löning L, Beier R, Ludwig WD, Ratei R, Harbott J, Boos J, Mann G, Niggli F,Feldges A, Henze G, Welte K, Beck JD, Klingebiel T, Niemeyer C, Zintl F, Bode U, Urban C, Wehinger H, Niethammer D, Riehm H, Schrappe M. **Risk adjusted therapy of acute lymphoblastic leukemia can decrease treatment burden and improve survival: treatment results of 2169 unselected pediatric and adolescent patients enrolled in the trial ALL-BFM 95.** Blood 2008;**111**(9):4477-4489. DOI: 10.1182/blood-2007-09-112920
8) Pui CH, Evans WE, Pharm. D. **Treatment of acute lymphoblastic leukemia.** N Engl J Med. 2006;**354**(2):166-178. DOI: 10.1056/NEJMra052603
9) Moghrabi A, Levy DE, Asselin B, Barr R, Clavell L, Hurwitz C, Samson Y, Schorin M, Dalton VK, Lipshultz SE, Neuberg DS, Gelber RD, Cohen HJ, Sallan SE, Silverman LB. **Results of the Dana-Farber Cancer Institute ALL Consortium Protocol 95-01 for children with acute lymphoblastic leukemia.** Blood 2007;**109**(3):896-904. DOI: 10.1182/blood-2006-06-027714
10) Pui Ching-Hon. "Acute lymphoblastic leukemia". Childhood Leukemias. New York: Cambridge University Press, 2012. 332-366. Print
11) Schultz KR, Pullen DJ, Sather HN, Shuster JJ, Devidas M, Borowitz MJ, Carroll AJ, Heerema NA, Rubnitz JE, Loh ML, Raetz EA, Winick NJ, Hunger SP, Carroll WL, Gaynon PS, Camitta BM. **Risk- and response- based classification of childhood B-precursor acute lymphoblastic leukemia: a combined analysis of prognostic markers from Pediatric Oncology Group (POG) and Children's Cancer Group (CCG).** Blood 2007;**109**:926-935. DOI: 10.1182/blood-2006-01-024729.
12) Smith M, Arthur D, Camitta B, Carroll AJ, Crist W, Gaynon P, Gelber R, Heerema N, Korn EL, Link M, Murphy S, Pui CH, Pullen J, Reamon G, Sallan SE, Sather H, Shuster J, Simon R, Trigg M, Tubergen D, Uckun F, Ungerleider R. **Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia**. J Clin Oncol 1996;**14**:18-24.
13) Pui CH, Campana D, Pei D, Bowman WP, Sandlund JT, Kaste SC, Ribeiro RC, Rubnitz JE, Raimondi SC, Onciu M, Coustan-Smith E, Kun LE, Jeha S, Cheng C, Howard SC, Simmons V, Bayles A, Metzger ML, Boyett JM, Leung W, Handgretinger R, Downing JR, Evans WE, Relling MV. **Treating childhood acute lymphoblastic leukemia without cranial irradiation.** N Engl J Med 2009;**360**:2730-2741. DOI: 10.1056/NEJMoa0900386.
14) Pui CH, Sandlund JT, Pei D, Campana D, Rivera GK, Ribeiro RC, Rubnitz JE, Razzouk BI, Howard SC, Hudson MM, Cheng C, Kun LE, Raimondi SC, Behm FG, Downing JR, Relling MV, Evans WE. **Improved outcome for children with acute lymphoblastic leukemia: results of Total Therapy Study XIIIB at St Jude Children's Research**

Hospital. Blood 2004;**104**:2690-2696. DOI:10.1182/blood-2004-04-1616

15) Conter V, Bartram CR, Valsecchi MG, Schrauder A, Panzer-Grümayer R, Möricke A, Aricò M, Zimmermann M, Mann G, De Rossi G, Stanulla M, Locatelli F,Basso G, Niggli F, Barisone E, Henze G, Ludwig WD, Haas OA, Cazzaniga G, Koehler R, Silvestri D, Bradtke J, Parasole R, Beier R, van Dongen JJ, Biondi A,Schrappe M. **Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: results in 3184 patients of the AIEOP-BFM ALL 2000 study.** Blood 2010;**115**:3206-14. DOI: 10.1182/blood-2009-10-248146.
16) Gaipa G, Basso G, Biondi A, Campana D. **Detection of Minimal Residual Disease in Pediatric Acute Lymphoblastic Leukemia. Cytometry Part B (Clinical Cytometry)** 2013;**84B**:359–369. DOI: 10.1002/cyto.b.21101
17) Borowitz MJ, Devidas M, Hunger SP, Bowman WP, Carroll AJ, Carroll WL, Linda S, Martin PL, Pullen DJ, Viswanatha D, Willman CL, Winick N, Camitta BM. **Children's Oncology Group. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: A Children's Oncology Group study**. Blood 2008;**111**:5477–5485. DOI: 10.1182/blood-2008-01-132837.
18) te Loo DM, Kamps WA, van der Does-van den Berg A, van Wering ER, de Graaf SS. **Prognostic significance of blasts in the cerebrospinal fluid without pleiocytosis or a traumatic lumbar puncture in children with acute lymphoblastic leukemia: experience of the Dutch Childhood Oncology Group.** J Clin Oncol 2006;**24**: 2332-6. DOI: 10.1200/JCO.2005.03.9727
19) Pui CH1, Campana D, Pei D, Bowman WP, Sandlund JT, Kaste SC, Ribeiro RC, Rubnitz JE, Raimondi SC, Onciu M, Coustan-Smith E, Kun LE, Jeha S, Cheng C, Howard SC, Simmons V, Bayles A, Metzger ML, Boyett JM, Leung W, Handgretinger R, Downing JR, Evans WE, Relling MV. **Treating childhood acute lymphoblastic leukemia without cranial irradiation**. N Engl J Med 2009;**360**:2730-41. DOI: 10.1056/NEJMoa0900386.
20) Matloub Y, Bostrom BC, Hunger SP, Stork LC, Angiolillo A, Sather H, La M, Gastier-Foster JM, Heerema NA, Sailer S, Buckley PJ, Thomson B, Cole C, Nachman JB, Reaman G, Winick N, Carroll WL, Devidas M, Gaynon PS. **Escalating intravenous methotrexate improves event-free survival in children with standard-risk acute lymphoblastic leukemia: a report from the Children's Oncology Group.** Blood 2011;**118**:243-51. DOI: 10.1182/blood-2010-12-322909.
21) Steinherz PG, Gaynon PS, Breneman JC, Cherlow JM, Grossman NJ, Kersey JH, Johnstone HS, Sather HN, Trigg ME, Uckun FM, Bleyer WA. **Treatment of Patients with Acute Lymphoblastic Leukemia with Bulky Extramedullary Disease and T-Cell Phenotype or Other Poor Prognostic Features**. Cancer 1998;**82**:600–12. DOI: 10.1002/(SICI)1097-0142(19980201)82:3<600::AID-CNCR24>3.0.CO;2-4
22) Möricke A, Zimmermann M, Reiter A, Gadner H, Odenwald E, Harbott J, Ludwig WD, Riehm H, Schrappe M. **Prognostic impact of age in children and adolescents with acute lymphoblastic leukemia: data from the trials ALL-BFM 86, 90, and 95**. Klin Padiatr 2005;**217**:310-20. DOI: 10.1055/s-2005-872515.
23) Dastugue N, Suciu S, Plat G, Speleman F, Cavé H, Girard S, Bakkus M, Pagès MP, Yakouben K, Nelken B, Uyttebroeck A, Gervais C, Lutz P, Teixeira MR, Heimann P, Ferster A, Rohrlich P, Collonge MA, Munzer M, Luquet I, Boutard P, Sirvent N, Karrasch M, Bertrand Y, Benoit Y. **Hyperdiploidy with 58-66 chromosomes in childhood B-acute lymphoblastic leukemia is highly curable: 58951 CLG-EORTC results**. Blood 2013;**121**:2415-23. DOI: 10.1182/blood-2012-06-437681.
24) Pulte D, Gondos A, Brenner H. **Improvement in survival in younger patients with acute lymphoblastic leukemia from the 1980s to the early 21st century.** Blood 2009;**113**:1408-11. DOI: 10.1182/blood-2008-06-164863
25) Nachman JB, La MK, Hunger SP, Heerema NA, Gaynon PS, Hastings C, Mattano LA Jr, Sather H, Devidas M, Freyer DR, Steinherz PG, Seibel NL. **Young adults with acute lymphoblastic leukemia have an excellent outcome with chemotherapy alone and benefit from intensive postinduction treatment: a report from the children's oncology group**. J Clin Oncol 2009;**27**:5189-94. DOI: 10.1200/JCO.2008.20.8959.
26) Pui CH, Pei D, Campana D, Bowman WP, Sandlund JT, Kaste SC, Ribeiro RC, Rubnitz JE, Coustan-Smith E, Jeha S, Cheng C, Metzger ML, Bhojwani D, Inaba H, Raimondi SC, Onciu M, Howard SC, Leung W, Downing JR, Evans WE, Relling MV. **Improved prognosis for older adolescents with acute lymphoblastic leukemia**. J Clin Oncol 2011;**29**:386-91. DOI:10.1200/JCO.2010.32.0325.
27) Boissel N, Auclerc MF, Lhéritier V, Perel Y, Thomas X, Leblanc T, Rousselot P, Cayuela JM, Gabert J, Fegueux N, Piguet C, Huguet-Rigal F, Berthou C, Boiron JM, Pautas C, Michel G, Fière D, Leverger G, Dombret H, Baruchel A. **Should adolescents with acute lymphoblastic leukemia be treated as old children or young adults? Comparison of the French FRALLE-93 and LALA-94 trials**. J Clin Oncol 2003;**21**:774-80. DOI: 10.1200/JCO.2003.02.053
28) Stock W, La M, Sanford B, Bloomfield CD, Vardiman JW, Gaynon P, Larson RA, Nachman J. **What determines the outcomes for adolescents and young adults with acute lymphoblastic leukemia treated on cooperative**

group protocols? A comparison of Children's Cancer Group and Cancer and Leukemia Group B studies. Blood 2008;**112**:1646-54. DOI: 10.1182/blood-2008-01-130237.

29) Pui CH, Boyett JM, Relling MV, Harrison PL, Rivera GK, Behm FG, Sandlund JT, Ribeiro RC, Rubnitz JE, Gajjar A, Evans WE. **Sex differences in prognosis for children with acute lymphoblastic leukemia**. J Clin Oncol 1999;**17**:818-24. PMID:10071272.
30) Shuster JJ, Wacker P, Pullen J, Humbert J, Land VJ, Mahoney DH Jr, Lauer S, Look AT, Borowitz MJ, Carroll AJ, Camitta B. **Prognostic significance of sex in childhood B-precursor acute lymphoblastic leukemia: a Pediatric Oncology Group Study**. J Clin Oncol 1998;**16**:2854-63. PMID:9704739
31) Chessells JM, Richards SM, Bailey CC, Lilleyman JS, Eden OB. **Gender and treatment outcome in childhood lymphoblastic leukaemia: report from the MRC UKALL trials**. Br J Haematol 1995;**89**:364-72. DOI: 10.1111/j.1365-2141.1995.tb03313.x
32) Bolufer P, Collado M, Barragán E, Cervera J, Calasanz MJ, Colomer D, Roman-Gómez J, Sanz MA. **The Potential Effect of Gender in Combination with Common Genetic Polymorphisms of Drug-Metabolizing Enzymes on the Risk of Developing Acute Leukemia**. Haematologica 2007;**92**:308-314. Doi:10.3324/haematol.10752.
33) Dorak MT, Lawson T, Machulla HKG, Darke C, Mills KI, Burnett AK. **Unraveling an HLA-DR Association in Childhood Acute Lymphoblastic Leukemia.** Blood 1999;**94**:694-700.
34) Khalifa NM, 2005, "The clinical significance of prognostic features in childhood acute lymphoblastic leukemia in South Egypt Cancer Institute", MD Thesis, South Egypt Cancer Institute, Assiut University, Assiut.
35) Möricke A, Zimmermann M, Reiter A, Henze G, Schrauder A, Gadner H, Ludwig WD, Ritter J, Harbott J, Mann G, Klingebiel T, Zintl F, Niemeyer C, Kremens B, Niggli F, Niethammer D, K Welte, Stanulla M, Odenwald E, Riehm H, Schrappe M. **Long-term results of five consecutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from 1981 to 2000.** Leukemia 2010;**24**:265-84. DOI:10.1038/leu.2009.257
36) Attarbaschi A, Mann G, Dworzak M, Wiesbauer P, Schrappe M, Gadner H. **Mediastinal mass in childhood T-cell acute lymphoblastic leukemia: significance and therapy response**. Med Pediatr Oncol. 2002;**39**:558-65. DOI 10.1002/mpo.10164.
37) Khalid S, Moiz B, Adil SN, Khurshid M. **Retrospective review of pediatric patients with acute lymphoblastic leukemia: A single center experience**. Indian J Pathol Microbiol 2010;**53**:704-10. DOI: 10.4103/0377-4929.72044.
38) Schultz KR, Pullen DJ, Sather HN, Shuster JJ, Devidas M, Borowitz MJ, Carroll AJ, Heerema NA, Rubnitz JE, Loh ML, Raetz EA, Winick NJ, Hunger SP, Carroll WL, Gaynon PS, Camitta BM. **Risk- and response-based classification of childhood B-precursor acute lymphoblastic leukemia: a combined analysis of prognostic markers from the Pediatric Oncology Group (POG) and Children's Cancer Group (CCG).** Blood 2007;**109**:926-935. DOI: http://dx.doi.org/10.1182/blood-2006-01-024729
39) Gaynon PS, Angiolillo AL, Carroll WL, Nachman JB, Trigg ME, Sather HN, Hunger SP, Devidas M . **Long Term Results of the Children's Cancer Group Studies for Childhood Acute Lymphoblastic Leukemia 1983–2002: a Children's Oncology Group Report.** Leukemia 2010;**24**:285–297. DOI:10.1038/leu.2009.262
40) Bürger B, Zimmermann M, Mann G, Kühl J, Löning L, Riehm H, Reiter A, Schrappe M. **Diagnostic cerebrospinal fluid examination in children with acute lymphoblastic leukemia: significance of low leukocyte counts with blasts or traumatic lumbar puncture.** J Clin Oncol 2003;**21**:184-8. DOI: 10.1200/JCO.2003.04.096
41) Sirvent N, Suciu S, Rialland X, Millot F, Benoit Y, Plantaz D, Ferster A, Robert A, Lutz P, Nelken B, Plouvier E, Norton L, Bertrand Y, Otten J. **Prognostic significance of the initial cerebro-spinal fluid (CSF) involvement of children with acute lymphoblastic leukaemia (ALL) treated without cranial irradiation: results of European Organization for Research and Treatment of Cancer (EORTC) Children Leukemia Group study 58881**. Eur J Cancer 2011;**47**:239-47. DOI: 10.1016/j.ejca.2010.10.019
42) Vaitkevičienė G, Forestier E, Hellebostad M, Heyman M, Jonsson G, Lähteenmäki PM, Rosthoej S, Söderhäll S, Schmiegelow K. **High white blood cell count at diagnosis of childhood acute lymphoblastic leukaemia: biological background and prognostic impact. Results from the NOPHO ALL-92 and ALL-2000 studies**. Eur J Haematol. 2011;**86**:38-46. DOI: 10.1111/j.1600-0609.2010.01522.x
43) Silverman B. Lewis. **"Acute Lymphoblastic Leukemia". Oncology of Infancy and Childhood: Expert Consult.** Ed. Stuart H Orkin, Ed. David E. Fisher, Ed. A. Thomas Look, Ed. Samuel E. Lux IV, Ed. David Ginsburg, Ed. David G. Nathan. Philadelphia: Saunders Elsevier, 2009. 297-330. Print.
44) Richards S, Pui CH, Gayon P**. Systematic review and meta-analysis of randomized trials of central nervous system directed therapy for childhood acute lymphoblastic leukemia.** Pediatr Blood Cancer 2013;**60**:185-95. DOI: 10.1002/pbc.24228.